Antibiotics ELISA kits available from ADI:

DE-100010	Clenbuterol ELISA kit, 96 tests (For Urine, Serum, Feed, Meat, Liver)
DE-100020	Ractopamine ELISA kit, (For Liver, Urine, Feed), 96 tests
DE-100030	Salbutamal ELISA kit, For Urine, Tissue, Feed, Animal Tissue, Aquatic, Honey, Intestine,, 96 tests
DE-100040	Chloramphenicol ELISA kit, 96 tests (For Animal Tissue, Aquatic, Honey, Intestine, Urine, Egg, Milk, Serum)
DE-100050	Florfenicol ELISA kit (For Animal Tissue, Aquatic, Honey), 96 tests
DE-100060	Nitrofuran (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofuran (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofuran (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofuran (AOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100090	Sulfonamides Residues (SAs) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests
DE-100100	Sulfamethazine (SM2) ELISA kit, 96 tests (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk)
DE-100110	Sulfamethoxydiazine (SMD) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine), 96 tests
DE-100120	Quinolones (QNS) ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100130	Enrofloxacin ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100140	Ampicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100150	Benzyl Penicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100160	Tylosin ELISA kit (For Meat, Liver, Honey, Egg), 96 tests
DE-100170	Trenbolone ELISA kit (For Animal Tissue, Aquatic, Urine), 96 tests
DE-100180	Diazepam ELISA kit (For Tissue, Urine, Feed), 96 tests
DE-100190	Diethylstilbestrol (DES) ELISA kit (Fish, Shrimp, Liver, Meat, Feed, Urine), 96 tests
DE-100200	Gentamicin ELISA kit (Chicken/Liver), 96 tests
DE-100210	Streptomycin ELISA kit, 96 tests (Chicken/Liver, Honey, Milk)
DE-100230	Olaquindox ELISA kit (Tissue) 96 tests
DE-100240	Sulfaquin-oxaline ELISA kit, (For Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests

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Instruction Manual No. M-DE-100170

Trenbolone ELISA KIT

Cat. # DE-100170

For Qualitative and Quantitative Determination of Trenbolone in animal tissue, aquatic and urine.

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Trenbolone ELISA KIT Cat. #DE-100170-TB

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100171
6x standard solution (1 ml each): 0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb	DE-100172
Enzyme conjugate (12 mL)	DE-100173
Antibody working solution (7 mL)	DE-100174
Substrate A solution (7 mL)	DE-SSA
Substrate B solution (7 mL)	DE-SSB
Stop solution (7 mL)	DE-ST
20x concentrated washing buffer (40 mL)	DE-WB
2× concentrated redissolving solution (50 mL)	DE-SS2
Instruction Manual	M-DE-100170

INTRODUCTION

Trenbolone is a steroid usually used by the veterinarians to increase muscle growth and appetite in animals. Veterinary implants trenbolone under the cattle's skin to increase its weight before sending them to slaughter. Its chemical formula Is $C_{18}H_{22}O_2$ and its molecular weight is 270.37 g/mol. It is available as trenbolone acetate, Valopharm USA sells it as Finaplix Gold and Quality Vet, Mexico sells it as TREMBOLONA QV75. It also comes in the form of trenbolone enanthate or trenbolone cyclohexylmethylcarbonate. Since its ability to increase muscle growth it is illegally used by bodybuilders and athletes. It will be active in the system for about 2 days, so it is injected once every two days. FDA has not approved the use of trenbolone in human.

Trenbolone has a very high binding affinity for the androgene receptor. Its affinity is three times higher as testosterone. After trenbolone is metabolized it increases the nitrogen uptake by muscles which lead to an increase of the protein synthesis rate. Another outcome of trenbolone uptake is the increase of appetite. Trenbolone reduce the amount of fat being deposited in the body and decrease the catabolism rate. Trenbolone is not metabolised by aromatase or 5-alpha reductase, which are enzymes used to break compounds down into estradiol or DHT. Due to this characteristic it does not retain any water like androgenic steroidal compounds such as testosterone or methandrostenolone do.

Side effects will include insomnia, high blood pressure, night sweats and increased libido. Female should avoid the intake of this drug even in small amounts because it will lead to virilization effects.

Alpha Diagnostic Intl's Trenbolone ELISA kit is a highly sensitive competitive type assay for the measurement of Trenbolone in animal tissue, aquatic and urine.

CALCULATION OF RESULTS

There are two methods to judge the results: the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Trenbolone concentration.

Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.310, and that of the sample Ⅱ is 0.820, the OD value of standard solutions is: 1.510 for 0 ppb, 1.320 for 0.05 ppb, 1.030 for 0.15 ppb, 0.660 for 0.45 ppb, 0.389 for 1.35 ppb , 0.198 for 4.05 ppb, accordingly the concentration range of the sample Ⅰ is 1.35 to 4.05 ppb, and that of the sample Ⅱ is 0.15 to 0.45 ppb (multiplied by the corresponding dilution fold).

Quantitative determination

The mean values of the absorbance values is obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average OD value of the sample or the standard solution
B_0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Trenbolone standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Trenbolone concentration in the sample. Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

Technical specifications

Sensitivity: 0.05 ppb

Detection limit

Meat, liver, shrimp, fish.....2.5 ppb
Urine.....0.25 ppb

Recovery rate

Meat, liver, shrimp, fish.....90 ±10%
Urine.....80 ±10%

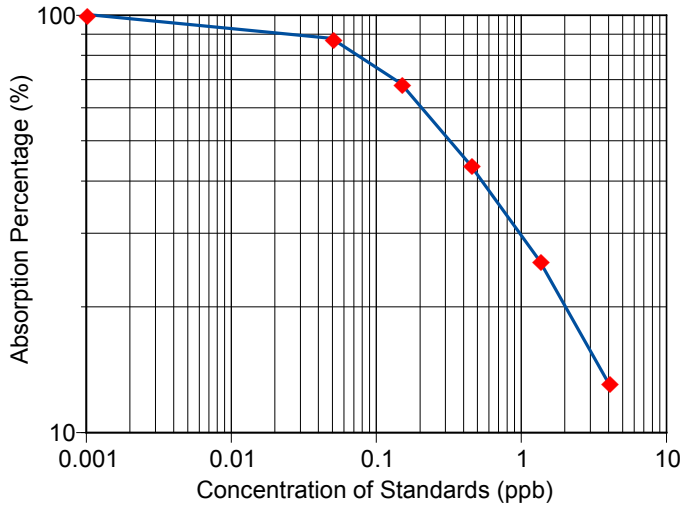
Cross-reaction rate

Trenbolone.....100%
Dehydroepiandrosterone(DHEA).....< 1%
Testosterone.....< 1%
Nandrolone.....4.5%
Methandienone.....< 0.1%

Work Sheet of Typical Assay-Trenbolone

Wells	Stds/samples	Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A** 0 ppb	1.510	100%
B1, B2	**Standard B** 0.05 ppb	1.320	87.42%
C1, C2	**Standard C** 0.15ppb	1.030	68.21%
D1, D2	**Standard D** 0.45 ppb	0.660	43.71%
E1, E2	**Standard E** 1.35 ppb	0.389	25.76%
F1, F2	**Standard F** 4.05 ppb	0.198	13.11%

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical assay Standard Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

This test kit is based on the competitive enzyme immunoassay for the detection of Trenbolone in the feed, urine, liver, meat, shrimp and fish. The conjugated antigens are pre-coated on the microwell stripes. The Trenbolone in the sample and the conjugated antigens pre-coated on the microwell stripes compete for the anti-Trenbolone antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with Trenbolone concentration in the sample. This value is compared to the standard curve and Trenbolone concentration is subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader, printer, mixer or stomacher, oscillator, centrifuge, nitrogen-drying device, measuring pipettes and balance (a sensibility reciprocal of 0.01 g)

Micropipettors: single-channel 20 to 100 µL and 200 to 1000 µL, and multi-channel 250 µL.

Reagents: NaOH, deionized water, Acetonitril (CH3CN), CHCl3, Glucuronidase/Arylsulfatase.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Trenbolone Kit is for research use only.

Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SAMPLE PRE-TREATMENT

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
2. Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment

1. 1 M NaOH: dissolve 0.4 g NaOH in deionized water to 100 mL.
2. The 2× concentrated redissolving solution is mixed with deionized water at 1:2(1 mL 2× concentrated redissolving solution+1 mL deionized water), for the treated sample redissolving.
3. CH3CN-0.1 M NaOH: mix 80 mL Acetonitrile and 20 mL 0.1 M NaOH.

Samples preparation

a) Animal tissue (meat, liver, fish, shrimp)

1. Take the tissue and feed sample, homogenize with stomacher.
2. Weigh 2±0.05 g of the homogenized sample, add 10 mL CH3CN-0.1 M NaOH, shake properly for 10 min, centrifuge at above 3000 r /min for 10 min.
3. Take 1 mL of the supernatant into centrifuge tube, add 0.5 mL 1 M NaOH, shake and add 5 mL CHCl3, shake for 2 min, centrifuge at above 3000 r /min for 10 min(heat for 2-4 min in 70 ℃ water if appearance of emulsifying, centrifuge again to make the lower be clear).
4. Remove the supernatant, take 1 mL clear liquid (the lower), blow to dry with nitrogen completely at 50 ℃.
5. Add 1 mL of the diluted redissolving solution to dilute dry residues.
6. Take 50 µL of the aqueous phase for further analysis.

Fold of dilution of the sample: 50

b) Urine

1. Put 2 mL sample into centrifuge tube, centrifuge at above 3000 r /min at room temperature (20-25℃) for 10 min, make urine be clear.
2. Transfer 1 mL of the supernatant into centrifuge tube, add 10 mL Glucuronidase/Arylsulfatase. Hydrolyze at 37 ℃ for 2 h, add 5 mL CHCl3 shake properly for 5 min, centrifuge at above 3000 r/min at room temperature (20-25 ℃) for 10 min, remove supernatant, take the clear phase (the lower layer), blow to dry nitrogen completely at 50 ℃.
3. Add 1 mL of the diluted redissolving solution to dissolve dry residues.
4. Take 50 µL, aqueous phase for further analysis.

Fold of dilution of the sample: 5

STORAGE AND STABILITY

Storage: store at 2 to 8 ℃, not frozen.

Expiration date: 12 months; date of production is on box.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25℃);
2. Return all reagents to 2-8℃ immediately after use;
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA;
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation Procedure

1. Take out all the necessary reagents from the kit and place at the room temperature (20-25 ℃) for at least 30 min. Note that each liquid reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.

3. Solution preparation: dilute 40 mL of the 20× concentrated washing buffer in the distilled or deionized water to 800 mL (or just to the required volume) for use.
4. Numbering: number the micro-wells according to samples and standard solution; each testing sample and standard solution should be performed in duplicate; record their positions.
5. Add 50 µL of the sample or standard solution to separate duplicate wells, add antibody working solution, 50 µL/well. Seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min.
6. Pour the liquid out of well, wash the microplate with the washing buffer at 250 µL/well for four to five times. Each time soak the well with the washing buffer for 10 s, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips)
7. Add enzyme conjugate, 100 µL/ well, seal the microplate with the cover membrane, react at 37 ℃ for 30 min
8. Continue as described in 6.
9. Coloration: add 50 µL of the substrate solution and then 50 µL of the B solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane and incubate at 37 ℃ for 15 min at dark for coloration.
10. Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (we recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

NOTES:

1. The room temperature below 20 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colorless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the coloration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 (A450 nm< 0.5) indicates its degeneration.
8. Coloration time is about 15 min, if the color is light , prolong the time of coloration but don't exceed 30 min.
9. The optimum reaction temperature is 37 ℃, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.